Interaction of fibronectin with arginine–agarose

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1. INTRODUCTION

Fibronectin, a high-M_r glycoprotein, interacts with many biological and biochemical substances (review [1–3]). The binding of fibronectin to gelatin allows its isolation by affinity chromatography [4]. Dissociation of fibronectin from gelatin–agarose by various amino compounds suggests the presence in fibronectin of specific binding sites for these compounds [5]. However, fibronectin has been reported to have very low affinity for arginine–agarose. For example, 0.1 M NaCl in 0.05 M Tris–HCl buffer eluted the fibronectin bound to arginine–agarose in [6]. We have confirmed these results in a study on the interaction between porcine blood plasma fibronectin and arginine–agarose with a relatively low content of arginine [7]. We have found that arginine–agarose with a higher arginine content retains >90% of fibronectin even when it is applied in the presence of 0.5 M NaCl.

This paper describes the binding of porcine plasma fibronectin to arginine–agarose, which can be dissociated with certain amino compounds, and the localization of the arginine-binding domains in fibronectin.

2. MATERIALS AND METHODS

2.1. *Preparation of affinity gels*

Gelatin–agarose was prepared as in [8]. Arginine was coupled to CNBr-activated Sepharose 4B as in [7]. Three preparations of arginine–agarose, I, II and III, were used, the arginine contents of which were 2.8, 2.0 and 0.7 mg/ml of wet gel, respectively, when determined by amino acid analysis.

2.2. *Preparation of fibronectin*

Porcine plasma fibronectin was prepared by affinity chromatography using gelatin–agarose and arginine–agarose III [7]. Pure fibronectin was recovered in the voided fraction from the latter affinity column.

2.3. *Assay of dissociation of fibronectin from arginine–agarose*

A solution of fibronectin (10 ml, 0.6 mg) in 0.1 M NaCl in 0.05 M Tris–HCl (pH 7.5) containing 1 mM EDTA and 20 mM ϵ-amino-caproic acid (buffer A) was applied on a column of arginine–agarose I (1 ml gel bed) at 4°C and washed with buffer A. Elution was effected with 10 ml of a test compound solution with various concentrations in buffer A and then washed with 10 ml of 0.1 M NaCl in buffer A. The fibronectin remaining bound to the column was eluted with 10 ml 1 M NaCl + 4 M urea in buffer A and 1 ml fractions were collected. Absorbance was read at 280 nm in each fraction and the concentration of fibronectin was determined using the absorbance value of 12.8 for 10 mg/ml [8]. On the basis of the value thus obtained, % elution effected with a test compound solution was calculated, and plotted against its concentration. From the resulting curve the concentration required to elute 50% of fibronectin from arginine–agarose I was determined.

In an experiment with dithiothreitol, fibronectin

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was pre-incubated in 0.1 M NaCl in buffer A containing 10 mM dithiothreitol at 4°C for 2 h, and applied to the column equilibrated with the same buffer.

2.4. *Arginine-binding domains*

Fibronectin was digested with cathepsin B [8] and was applied to arginine–agarose I at 4°C. It was washed with 0.1 M NaCl in buffer A, and then elution was effected with increasing concentrations of NaCl in buffer A. The fractions thus separated were monitored by reading the absorbance at 280 nm, and were subjected to SDS–polyacrylamide gel electrophoresis as in [8].

2.5. *Modification of arginine in gelatin–agarose*

The guanidinium groups of gelatin–agarose were modified as in [9] with 50 mM butane-2,3-dione at 25°C for 24 h in 50 mM sodium borate buffer (pH 7.5) containing 0.15 M NaCl (buffer B). Subsequent amino acid analysis showed that 83% of arginine had been modified. A solution of fibronectin was dialyzed against buffer B, and then was applied to this column at 4°C. After the column was washed with buffer B, the bound fibronectin was eluted with 1 M NaCl–4 M urea in buffer B, and determined as above.

3. RESULTS AND DISCUSSION

Although fibronectin prepared by gelatin–agarose affinity chromatography was of considerable purity, chromatography with arginine–agarose III was necessary to remove another gelatin-binding protein [7] which has been designated polynectin [10]. Fibronectin was recovered in the non-binding fraction from this arginine column in the presence of 0.1 M NaCl in 0.05 M Tris–HCl buffer. However, both arginine–agarose preparations I and II could retain 90–93% of fibronectin applied under the same conditions. The difference in binding seemed to be due to the difference in the arginine content in the affinity gels. Fibronectin was reported not to bind to arginine–agarose prepared in [6] under similar conditions. In their report, no data are found regarding the arginine content in the gel.

Table 1 shows the concentration of various compounds which brings about 50% elution of fibronectin bound to arginine–agarose I. Spermidine was found to be the most effective eluent. Diaminocompounds and monoamino–monohydroxy compunds also dissociated the interaction. Certain spatial arrangements of two functional groups appear to be important for effective dissociation, since, for example, diaminohexane is much more effective than diaminoethane. Dissociation by ornithine and lysine was less effective. Therefore, the fibronectin–arginine interaction seems to be specific.

Table 1

Concentration required to elute 50% of fibronectin bound to arginine–agarose I at pH 7.5 and 4°C

Compound	Conc. (M)
Spermidine	0.18
Arginine	0.34
1,6-Diaminohexane	0.36
2-Aminoethanol	0.37
1-Amino-2-propanol	0.39
Putrescine	0.42
Guanidine	0.48
Glucosamine[a]	0.53
1,2-Diaminoethane	0.75
Ornithine	0.99
Lysine	>1
Glycine	>1
Proline	>1
Urea	3.1

[a] A solution, the pH of which was adjusted to 6.5, was used, because of gradual coloration at pH 7.5

Only a slight dissociation (8%) was brought about with 0.5 M NaCl. Even with 1 M NaCl, only 51% of fibronectin was eluted. This result suggests that the arginine–fibronectin interaction was not of simple ionic nature. Dissociation was almost complete (96%), when 1 M urea was added to 1 M NaCl.

In the presence of 10 mM dithiothreitol, 89.4% of fibronectin was still bound to arginine–agarose I. Thus, a three-dimensional conformation maintained by disulfide bonds of fibronectin seems to be of little importance for its interaction with arginine–agarose. A preliminary experiment with reduced and carboxymethylated fibronectin supported this view (unpublished). This is in contrast to a report which showed that native disulfide

bridges are essential in fibronectin–gelatin interaction [11]. Because as much as 89.3% of fibronectin was bound to gelatin–agarose with 83% modified arginine under the condition where 90.7% was bound to unmodified gelatin–agarose, the arginine residues in gelatin are presumably not essential in this interaction. It seems therefore, that the interactions with gelatin and with arginine occur at different sites of fibronectin.

Porcine liver cathepsin B is known to cleave fibronectin into limited numbers of fragments containing ligand specific domains [8]. When a cathepsin B digest was separated by affinity chromatography with arginine–agarose I, several fractions were obtained as shown in fig. 1. Each fraction was examined by SDS gel electrophoresis (fig. 2). Of the 5 major fragments [8], heparin-binding M_r 30000 fragment and gelatin-binding M_r 50000 fragment interacted with arginine–agarose I only weakly under a condition similar to one obtained with physiological saline (fig. 2, gels 2 and 3). The other 3 fragments with M_r 130, 150 and 175 $\times 10^3$ were bound to arginine–agarose I (fig. 2, gels 4–6) under this condition. These findings suggest that the binding activities of fibronectin to gelatin–agarose and to arginine–agarose are two different expressions of its multifunctional nature.

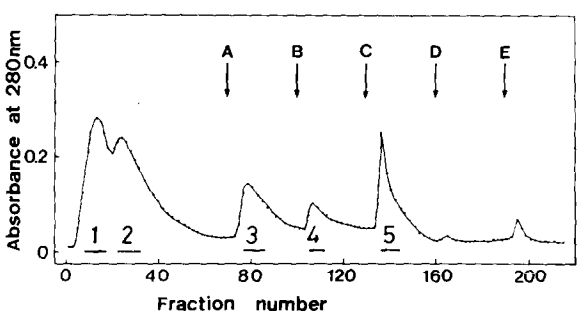

Fig. 1. Fractionation of cathepsin B fragments of porcine plasma fibronectin by affinity chromatography with arginine–agarose I. A solution of the digest derived from 26 mg fibronectin was applied to an arginine column (1.6 × 4.5 cm). The column was washed with 0.1 M NaCl in buffer A and stepwise elution was effected with 0.15 M NaCl, 0.3 M NaCl, 0.5 M NaCl, 1 M NaCl, and 1 M NaCl plus 4 M urea in buffer A as shown by arrows A–E, respectively, and 3.2 ml fractions were collected. Total recovery was 97% in terms of absorbance at 280 nm.

While this manuscript was in preparation, Vartio reported the localization of cathepsin G fragments of human plasma fibronectin which bind to arginine–CH-Sepharose 4B [12]. The localization of the major arginine-binding fragment in [12] appears to correspond to the region of M_r 30000–50000 in our experiment, since it has a gelatin-binding activity. Another arginine-binding site located carboxyl-terminally to the gelatin-binding fragment was suggested [12]. The interac-

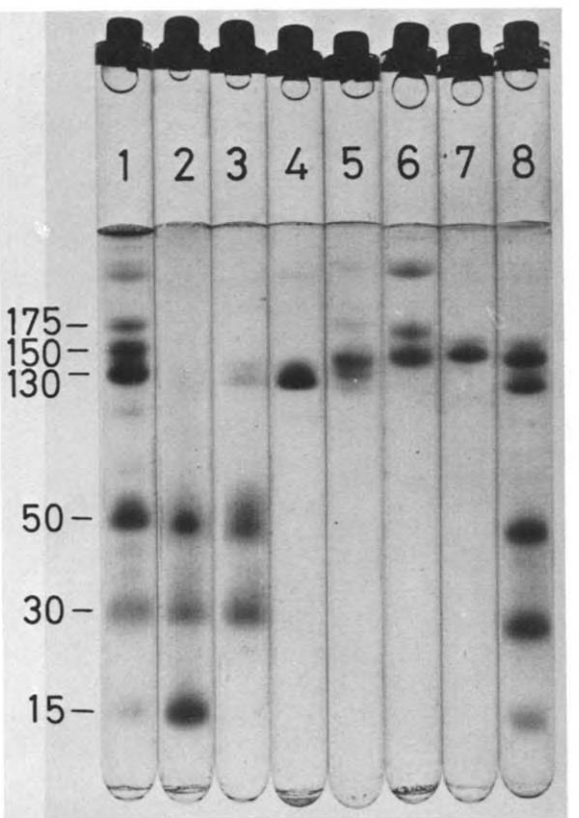

Fig. 2. SDS–polyacrylamide gel electrophoresis of the fractions of affinity chromatography with arginine–agarose I. Fractions indicated by bars 1–5 in fig. 1 were collected and concentrated, if required, and analyzed using 5% gels [8]: (1) total cathepsin B digest; (2–6) fractions 1–5; (7) fraction 5, reduced; (8) total cathepsin B digest, reduced. Note disappearance of the M_r 175000 fragment in gels 7 and 8 under the reduced condition in accordance with [8]. The estimated molecular masses [8] are indicated $\times 10^{-3}$. Gels were stained with Coomassie brilliant blue R-250.

tion in the experiment of [12], however, was very weak and could be dissociated with 0.1 M NaCl in 5 mM Tris buffer [12].

Our data suggest that the major arginine-binding sites of fibronectin are not in the gelatin-binding M_r 50000 fragment, but in the M_r 130000 and 150000 fragments. Since these large fragments are considered to contain cell-binding sites [3,8], the present study raises a possibility that some cells might interact with fibronectin through a protein which contains arginine residues occupying the sites favourable for the interaction with this glycoprotein to take place.

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